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(54) High throughput polymorphism screening

(57) Methods are provided for determining the identity of a polymorphic nucleotide in a complex mixture of nucleic acids where one or more distinct polymorphisms can be present in the mixture, and multiple polymorphisms can be screened in parallel. Target nucleic acids are amplified using bridge amplification techniques. The detection and identification of the specific polymorphic residue(s) is based on readout methods that utilize the specificity of specific enzymes for complementary DNA sequences. These approaches result in a labeled nucleotide covalently attached to the amplicon, where the identity of the nucleotide is informative of the polymor-

phic sequence. In one aspect, the readout process uses primer extension protocols, where the specific base incorporated by DNA polymerase is determined by the sequence at the polymorphic site. In another aspect, the identity of a specific base hybridized and ligated to the amplicon is determined by the sequence at the polymorphic site. The polynucleotide to which the label has been attached can be detected *in situ*, i.e. bound to the solid substrate used for amplification; or can be released and detected.

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	<213> homo sapien	
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Claims

1. A method of determining the identity of a polymorphic nucleotide, said method comprising:
 30 contacting under hybridizing conditions, a target nucleic acid comprising a polymorphic site, and a solid substrate comprising one or more bound locus-specific primer pairs;
 amplifying said target nucleic acid with said locus-specific primer pair, wherein said amplifying results in an amplification product bound to the solid support at each end;
 35 contacting said amplification product with a labeled probe comprising at least one detecting nucleotide that will specifically base pair with said polymorphic nucleotide, in the presence of an enzyme that catalyzes the formation of a covalent bond between said detecting nucleotide and said amplification product; and
 detecting said label;
 wherein the identity of the label on said detecting nucleotide indicates the complement of the polymorphic nucleotide.
2. The method according to Claim 1, wherein said enzyme is DNA polymerase.
3. The method of claim 2, wherein said amplification product is denatured and contacted with an extension primer that hybridizes to a site immediately adjacent to said polymorphic nucleotide, prior to contacting with said labeled probe, wherein DNA polymerase extends from said extension primer to covalently attach a labeled probe to its 3' end.
4. The method of Claim 2, wherein said amplification product is cleaved with an endonuclease to generate a free end; and
 50 cleaving with a distance-cleaving endonuclease, resulting in a cleavage product having an overhang strand and a recessed strand comprising a 3' terminus, wherein the polymorphic nucleotide is on the single-stranded overhang of the cleavage product, wherein said recessed strand provides an extension primer for said DNA polymerase.
5. The method of claim 2, wherein said amplification product is contacted with a plurality of labeled probes selected from the group consisting of at least two differentially labeled dideoxynucleotides.

6. The method of claim 3, wherein a plurality of mutually distinguishable extension primers are used.
7. The method of Claim 1, wherein said enzyme is ligase.
- 5 8. The method of claim 7, wherein said amplification product is cleaved with an endonuclease to generate a free end; and
10 cleaving said amplification product with a distance-cleaving endonuclease, resulting in a cleavage product having a single-stranded overhang strand and a recessed strand, wherein the recessed strand has a 3' terminus, wherein the polymorphic nucleotide is on the single-stranded overhang of the cleavage product, wherein said recessed strand; contacting with ligase and at least one nucleotide complementary to said polymorphic nucleotide under conditions that permit covalent linkage.
9. The method of claim 8, wherein said amplification product is contacted with a plurality of differentially labeled oligonucleotide probes selected from the group consisting of all possible sequences of said single-stranded overhang.
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10. The method of claim 10, wherein at least two different labels are used.
11. The method of claim 8, wherein the polymorphic nucleotide is the first nucleotide on the single-stranded overhang
20 of the cleavage product.
12. The method of Claim 1, wherein said amplification product comprising said detecting nucleotide is released from said substrate for detection.
- 25 13. The method of Claim 1, wherein said amplification product comprising said detecting nucleotide is detected *in situ*.
14. The method according to Claim 1, wherein said solid substrate comprises a capture primer.

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